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SEROTONERGIC INNERVATION OF THE LOCUS COERULEUS FROM THE DORSAL RAPHE AND ITS ACTION ON RESPONSES TO NOXIOUS STIMULI

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SUMMARY

The connexions between the dorsal raphe nucleus and the nucleus locus coeruleus were studied in urethane anaesthetized rats.

1. Cells in the locus coeruleus gave an excitatory response to a noxious stimulus, e.g. leg pinch.

2. This excitatory response was blocked by either a parenteral or an ionophoretic injection of morphine and recovered after an injection of naloxone.

3. Electrical stimulation in the region of the dorsal raphe blocked excitatory locus coeruleus responses to noxious stimuli.

4. While naloxone did not antagonize the effects of the dorsal raphe stimulation towards locus coeruleus activity, these effects were absent in rats pretreated with a serotonin synthesis inhibitor, PCPA or with 5,7-DHT which destroys serotonin-containing terminals, and were reduced by the serotonin antagonist methysergide.

5. A serotonin-containing inhibitory pathway between the dorsal raphe and the locus coeruleus is proposed to account for these results.

INTRODUCTION

The analgesic properties of electrical stimulation of the mid-brain raphe nuclei have been investigated intensively in recent years (Mayer & Price, 1976; Oliveras, Besson, Guilbaud & Liebeskind, 1974). The involvement of serotonin (5-hydroxytryptamine, 5-HT) in stimulation-produced analgesia was suggested by a number of investigators (Akil & Liebeskind, 1975; Way, 1972). The recent discovery of endogenous brain morphine-like peptides (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) lent intuitive validity to the phenomenon of stimulation-produced analgesia; stimulation might cause a release of the endogenous peptides at receptor sites involved in pain transmission. In spite of its appeal, this hypothesis has not gained much experimental support as yet; there is no clear correlation between the regional distribution of morphine receptors (and high enkephalin concentrations) and sites where analgesia is produced by electrical stimulation or topical morphine application (Pert, Kuhar & Snyder, 1975; Yaksh, Yeung & Rudy, 1976; Simantov, Kuhar & Pasternak, 1976; Oliveras *et al.* 1974). The dorsal raphe nucleus and the adjacent periaquaductal area do not contain high enkephalin concentrations, yet their stimulation produces potent analgesia. Curiously, it is reported that cells in dorsal raphe do not respond to noxious stimuli or to morphine (Haigler, 1976; Korf,

Bunney & Aghajanian, 1974). It is therefore plausible that the analgesic properties of dorsal raphe stimulation are exerted elsewhere in the brain, and that the dorsal raphe does not participate naturally in pain processes. A possible site of interaction between raphe stimulation and nociception is the nucleus locus coeruleus. It contains a high concentration of serotonin (Palkovits, Brownstein & Saavedra, 1974) possibly of dorsal raphe origin (Sakai, Touret, Salvert, Leger & Jouvet, 1977; Taber-Pierce, Foote & Hobson, 1976) as well as a high concentration of morphine receptors (Pert *et al.* 1975).

There is evidence to suggest a functional interaction between the dorsal raphe and the locus coeruleus (Kostowski, Samanin, Bareggi, Marc, Garattini & Valzelli, 1974; Kostowksi, 1975; Lewis, Renaud, Buda & Pujol, 1976). Electrical stimulation of the locus coeruleus produces analgesia (Segal & Sandberg, 1977). Unlike the case for the dorsal raphe, there is a direct projection from the locus coeruleus to spinal cord (Basbaum & Fields, 1977).

Cells in the locus coeruleus respond to noxious stimuli by excitation and to morphine by inhibition (Korf *et al.* 1974; Bird & Kuhar, 1977) and locus coeruleus lesion reduces morphine analgesia (Sasa, Munekiyo, Osumi & Takaori, 1977). The present study was therefore undertaken to investigate the connexion between the dorsal raphe and cells of the locus coeruleus in the rat, in an attempt to clarify the possible interaction between serotonin, noradrenaline, and the endogenous morphine-like peptides in the brain.

METHODS

Forty-eight adult male Wistar rats of a local breeding colony were used for the physiological experiments. Additional rats were used for the anatomical study. Rats in this study were anaesthetized with chloral-hydrate (350 mg/kg). Using ionophoresis, horseradish peroxidase (HRP) was ejected from a glass pipette into the region of the locus coeruleus. After a survival period of 24 hr, the rat was perfused and the brain processed as described previously (Segal, 1977). Rats were anaesthetized for the physiological study with urethane (1·2 g/kg) and placed in the stereotaxic frame. A concentric bipolar stimulating electrode was lowered into the region of the dorsal raphe nucleus (5·0–6·5 mm below lambda, with skull levelled between bregma and lambda). Recording of single locus coeruleus extracellular activity was made through a glass micropipette filled with 0·1 % fast green dye in 3 M-NaCl or the centre barrel of a five-barrel micropipette. The DC resistance of the recording electrode was 0·5–2 MΩ in both cases. The recording electrode was directed towards the locus coeruleus at a 40° angle, from the posterior suture, 1·1 mm lateral to the mid line in order to avoid crowding of the stimulating and recording electrodes. Locus coeruleus cells were tentatively identified during recording on the basis of (1) depth (approx. 6·5 mm below skull), just under the cerebellum, and (2) slow spontaneous firing (0·1–1 spikes/sec) rates, as previously reported (Korf *et al.* 1974; Nakamura, 1977). Occasionally activity of cells with large amplitude spikes were recorded in the proper depth. These cells reacted to small movements of the lower jaw and were thought to belong to the mesencephalic nucleus of the trigeminus. Upon detection of these cells the electrode was withdrawn and placed 0·1 mm medially. The typical locus coeruleus-like cellular activity was then recorded. Routinely there were up to two recording-electrode penetrations in each hemisphere, to avoid mistakes in later localization of the electrodes in the tissue. A characteristic feature of 'locus coeruleus neurones' was their excitatory reaction to a noxious stimulus, e.g. a pinch. The pinch was applied routinely to the hind leg, with a haemostat closed to the first notch for approx. 3 sec each time. The dorsal raphe was stimulated at a rate of 10–20 pulses/sec with pulses of 0·1 msec, 3–4 V with a Digitimer battery-operated stimulator. Further details of the methodology of recording, data analysis and ionophoresis are presented elsewhere (Segal, 1976*a*, *b*; Segal & Bloom, 1974). The following drugs were used for ionophoresis: methysergide maleate (MS 0·1 M, Sandoz pH 5·0), morphine-HCl (Mo, 0·1 M, Asia Co. pH 4·7), naloxone-HCl (Nal, 0·1 M, Endo

Labs pH 5·5), serotonin creatinine sulphate (5-HT, 0·1 M, Aldrich pH 5·0). The following drugs were applied parenterally: Mo (10 mg/kg I.P.), MS (2–10 mg/kg), NaI (2 mg/kg I.P.), DL-p-chlorophenylalanine-HCl (PCPA, Merck, 350 mg/kg I.P.), 5-hydroxytryptophane (5-HTP, Merck, 30–60 mg/kg I.P.), 5,7-dihydroxytryptamine (5,7-DHT, 5 μg in 1 μl. saline injected stereotoxically into the region of the dorsal raphe 5–7 days before the experiment).

At the end of the recording session, current was applied to some recording electrodes to eject fast-green out of the pipette to ease later histological verification of the electrode tip. In all cases the rat was perfused at the end of the experiment with a mixture of 1·5% glutaraldehyde and 1·5% paraformaldehyde and the brain sectioned on a freezing stage. The stimulating and recording electrodes were later localized in the cresyl violet-stained sections (Pl. 1).

RESULTS

The dorsal raphe projection to the locus coeruleus

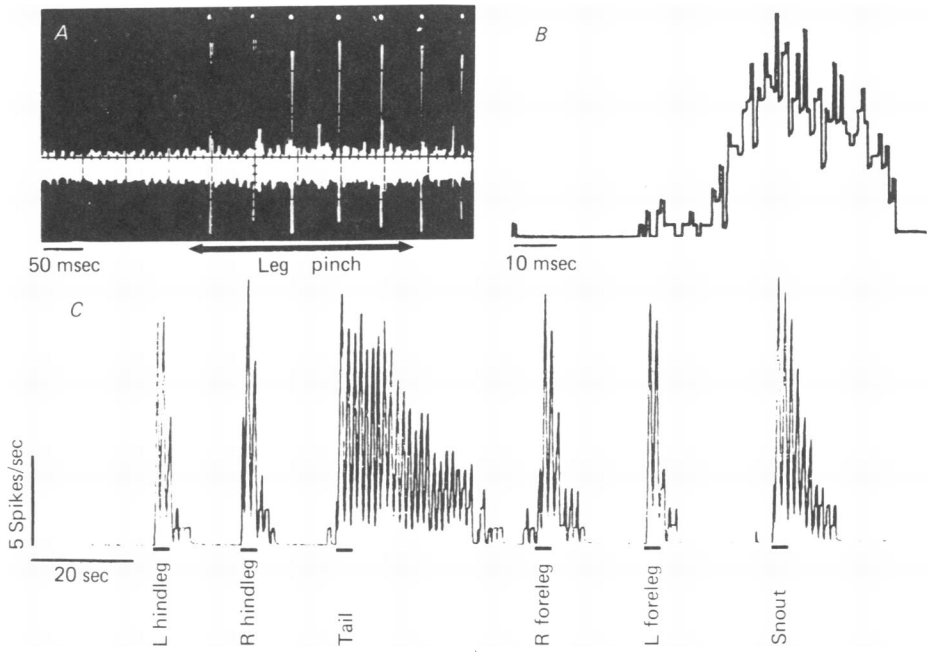
For the anatomical study, only brains where the injection of HRP resulted in a restricted staining of the main nucleus locus coeruleus were selected for analysis (Pl. 2). Brains in which some of the HRP may have leaked into the fourth ventricle were excluded. In the former group (five brains) a search was made for neurones in the central grey, periventricular and periaquaductal regions, that would demonstrate the characteristic retrograde labelling of HRP. Such neurones were found mainly in the ipsilateral aspects of an intermediate cell group, between the dorsal and ventral cell groups of the dorsal raphe (Pl. 2). Labelled neurones were not found in the median raphe or other components of the raphe nuclei.

Responses to noxious stimuli

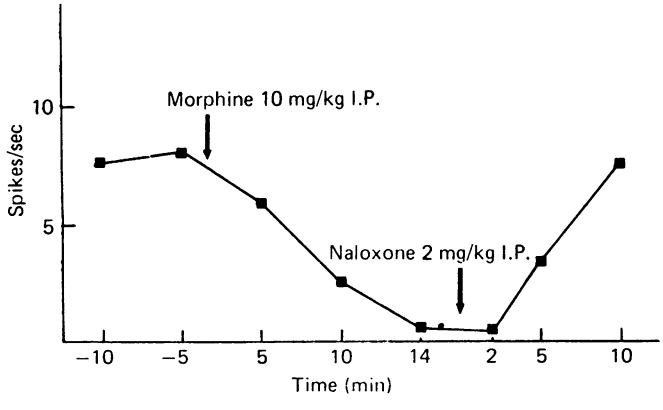
Activity of fifty-five neurones in the region of the locus coeruleus was recorded in forty-five rats. Most cells (thirty-five) had a low (less than 1 spike/sec) and irregular firing frequency (Nakamura & Iwama, 1975). Eleven more cells fired at a rate of approximately 1–5 spikes/sec and nine more cells fired at a higher rate (6–20 spikes/sec).

Most cells were localized in the anterior region of the locus coeruleus. Some electrode tips were found just rostral to the main nucleus locus coeruleus.

A characteristic feature of locus coeruleus cells was their response to a noxious stimulus. Most cells emitted a burst of 5–10 spikes which outlasted the duration of the pinch. An inhibitory interval occasionally followed the initial excitatory response. The response was not specific to a certain region, and could be seen with a pinch applied to the hindlegs, forelegs, tail or snout. A needle prick to the abdomen, upon injection of a drug, also caused the cell to fire. The response was specific to a noxious stimulus; light stroke of parts of the body did not result in cell firing. Other modalities were not tested systematically in this experiment. All thirty-five slow-firing cells (0·01–1 spikes/sec) reacted to the noxious stimulus with an excitatory response. An inhibitory response was dominant in the cells with the higher spontaneous firing frequency; nine of eleven cells with 1–5 spikes/sec reacted with an excitatory response and two with an inhibitory response. Of the nine cells with a spontaneous firing of 6-20 spikes/sec, three reacted with excitation and six with an inhibitory response.



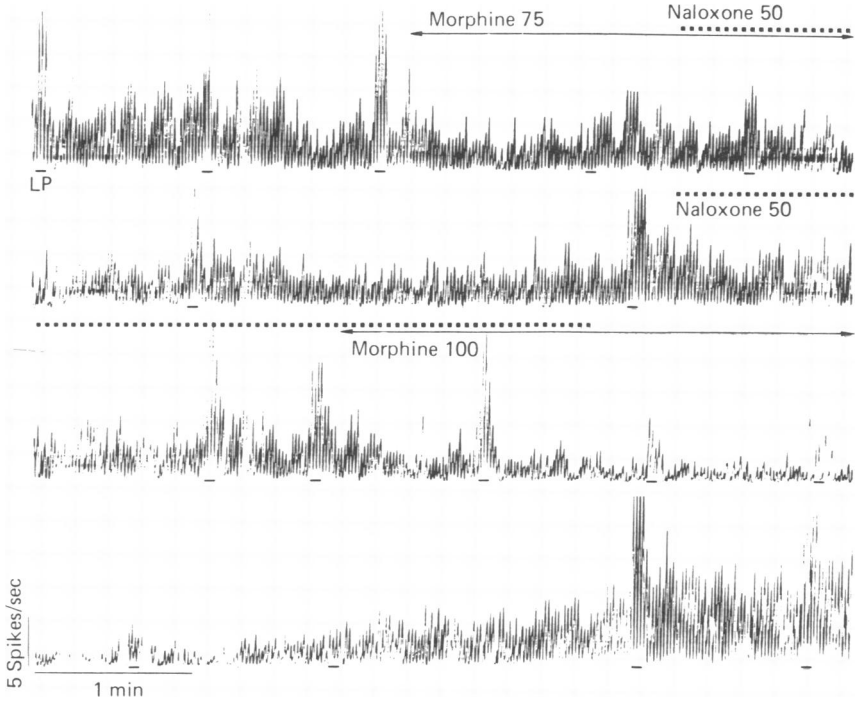
Text-fig. 1. Responses of a locus coeruleus cell to noxious stimuli, *A*, a specimen record of response of the neurone to a leg pinch. The dots above the cellular spikes represent the output of the waveform discriminator. *B*, an interspike interval histogram of the cell in *A*, summing 1024 intervals. A fairly regular firing pattern is seen during and after application of the pinch. *C*, responses of the neurone to noxious stimuli applied to various parts of the body; left and right hind legs, tail, right and left forelegs and snout. The cellular activity is integrated over 1 sec intervals. The bars underneath the cumulative records in this and the following records indicate the duration of the pinch (usually 3 sec). The differences in the magnitudes of the responses in this Figure do not represent a systematic difference in the efficacy of any noxious stimuli.



Text-fig. 2. The effects of morphine and naloxone on locus coeruleus cellular excitatory responses to a leg pinch. The magnitude of the responses is plotted on the ordinate in spikes/sec. The value plotted represents the response at the peak (the largest 1 sec interval rate). The spontaneous activity of the cell was low (0·01–0·1 spikes/sec). An injection of morphine caused a reduction of the response within 10–15 min. After injection of naloxone (2 mg/kg I.P.) a recovery of the excitatory response to the pinch was seen.

Effects of morphine

The effects of parenteral injections of morphine on locus coeruleus cellular responses to pinch were tested with six cells. An injection of morphine, 10 mg/kg, reduced the spontaneous activity of the cells, as previously reported (Korf *et al.* 1974) but also blocked the excitatory response to the noxious stimulus in all six cells tested within 10–20 min (Text-fig. 2). Upon injection of naloxone (in all five cells tested), there was a rapid recovery of the excitatory response to the pinch, as well as an increase in the spontaneous activity, back to the pre-morphine rates.



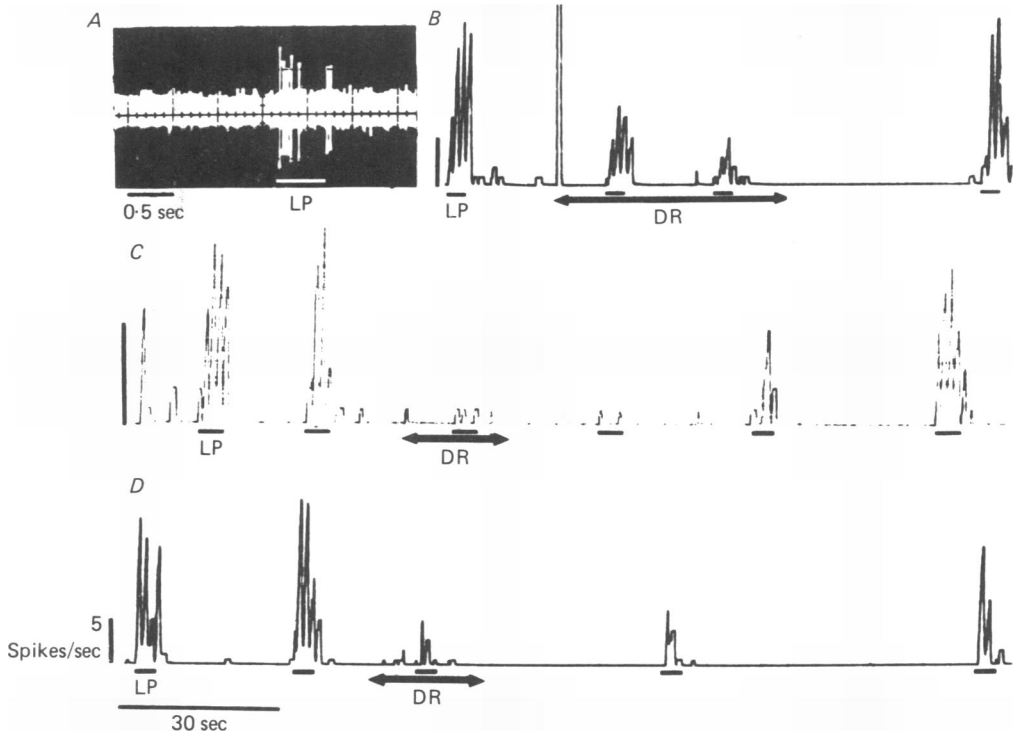
Text-fig. 3. The effects of morphine applied ionophoretically on cellular responses to a noxious stimulus. The four traces are a continuous, cumulative record of the activity of a cell in the region of the locus coeruleus. Leg pinch (LP) induced an excitatory response of the cell which was suppressed during iontophoretic application of 75 nA of morphine (top record). Concurrent ionophoresis of naloxone caused a partial recovery of the response (top record). Ejection of naloxone (second record) counteracted the effects of morphine (second and third records), although it did not have any effect by itself. When naloxone was retained in the pipette (third record) and morphine ejected alone, it suppressed spontaneous and pinch-induced activity of the neurone (fourth record).

In order to test the possibility that morphine may exert its action directly on locus coeruleus cells, it was applied ionophoretically via the five-barrel micropipette. In ten of thirteen cells tested, the spontaneous activity, as well as the excitatory responses to the hind leg-pinch were reduced during the ionophoretic application of morphine. This effect was rather long lasting after termination of the ejection current of morphine (4–5 min, Text-fig. 3). Naloxone, applied ionophoretically, antagonized

the effects of morphine on both spontaneous and evoked locus coeruleus activity in four cells tested (Text-fig. 3).

Effects of dorsal raphe stimulation

The effects of low current (20–50 μA) electrical stimulation in the region of the dorsal raphe nucleus were assessed with twenty-two cells in the locus coeruleus. The stimulation caused a reduction of the spontaneous activity of most cells tested (fourteen cells) while not affecting the activity of the other cells tested. Due to low spontaneous activity of these cells, the exact latency of the inhibitory responses to single-pulse stimulation of dorsal raphe could not be determined. Higher currents (100–200 μA) occasionally caused the appearance of driven cellular responses

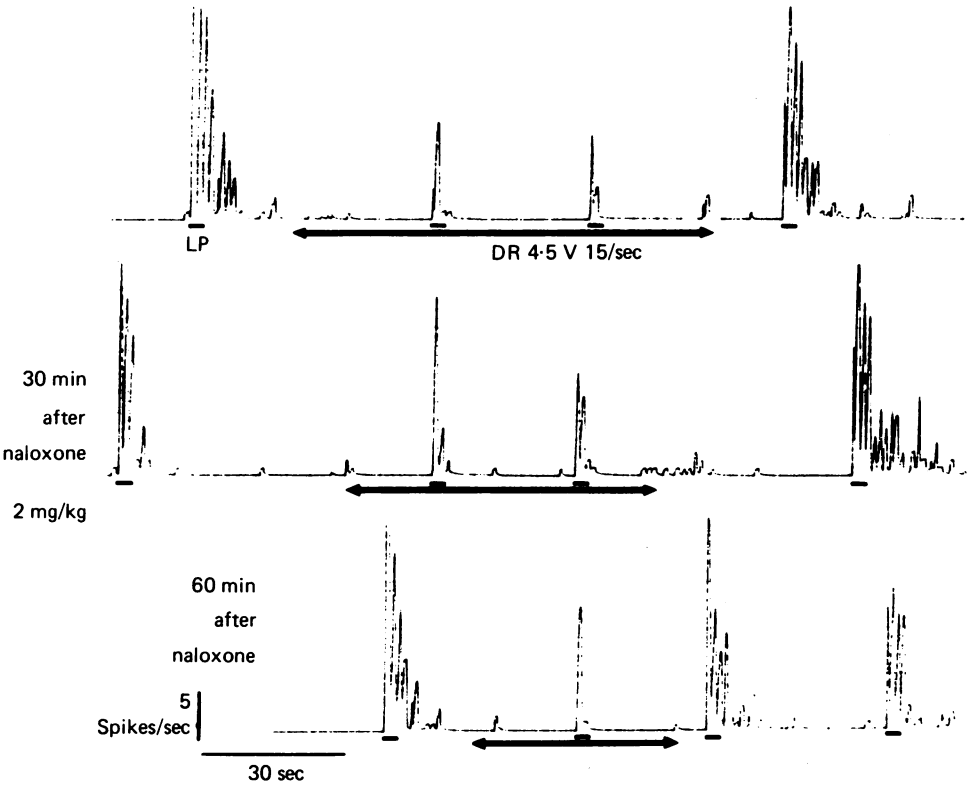


Text-fig. 4. The suppressing action of dorsal raphe (DR) stimulation on leg pinch (LP) induced locus coeruleus excitation in three different (*A*, *B*, *C*, *D*) neurones. *A*, a specimen record of locus coeruleus cellular responses to leg pinch. *B*, *C*, *D*, records of three cells. In all three, dorsal raphe stimulation suppressed responsiveness of the neurones to leg pinch. Note the difference in effectiveness of stimulation in the three records. This difference was not a function of the pre-stimulation magnitude of the responses of the locus coeruleus neurones.

accompanied by an evoked field potential in the locus coeruleus with a latency of 3–5 msec. Irrespective of the ability of low current dorsal raphe stimulation to reduce spontaneous activity of the neurones, it blocked locus coeruleus cellular responses to the noxious stimuli (Text-fig. 4). This effect was fairly similar to that of morphine in that it outlasted the period of stimulation. With slow-firing cells the effect of the

stimulation on spontaneous activity was not obvious although the blockade of the responses to the pinch was marked. The spontaneous activity of cells with higher firing frequencies was reduced by the stimulation yet in only one of five of these cells did dorsal raphe stimulation block responses to pinch.

In order to test the possibility that dorsal raphe action is mediated by a morphine-receptor, an attempt was made to antagonize the inhibitory action of dorsal raphe stimulation by naloxone (five cells) (Text-fig. 5). Such attempts have failed; although some antagonism of dorsal raphe stimulation effects could occasionally be recorded, this was neither consistent nor profound.

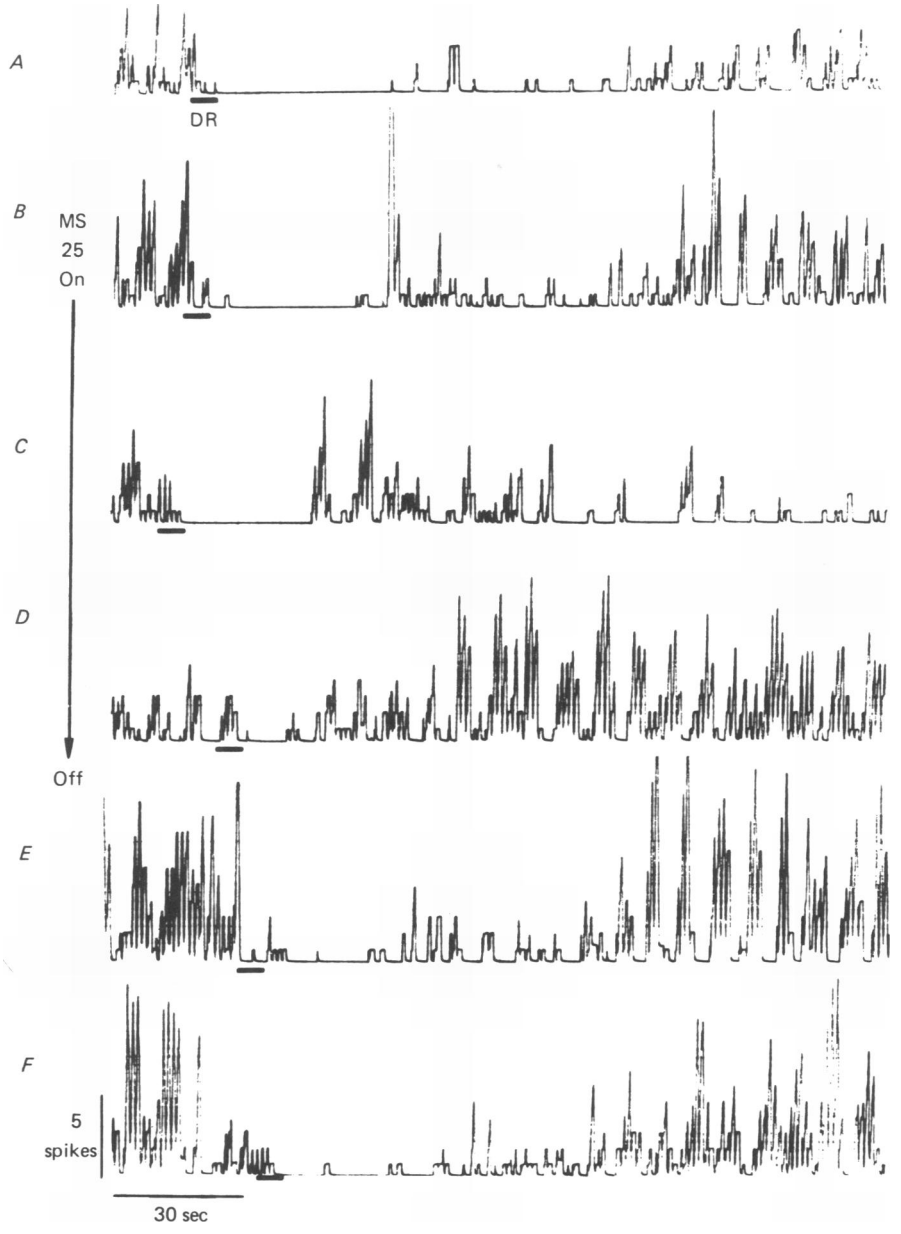


Text-fig. 5. Absence of naloxone (2 mg/kg I.P.) antagonism of the dorsal raphe stimulation effects. The three traces are from a continuous record of locus coeruleus responses to the leg pinch. It should be noted that naloxone did not affect the magnitude of these responses to the noxious stimulus and it had little effect towards dorsal raphe induced suppression of the responses.

Effects of serotonergic drugs

The serotonergic basis of the dorsal raphe inhibitory effects was tested by using a serotonin antagonist; methysergide. When applied by ionophoresis, methysergide had at least a partial antagonistic action towards locus coeruleus responses to dorsal raphe stimulation in seven of fourteen cells tested (Text-fig. 6). The inhibitory action of dorsal raphe stimulation towards the other seven cells was not affected by methysergide. It should be pointed out that methysergide exerted direct effects

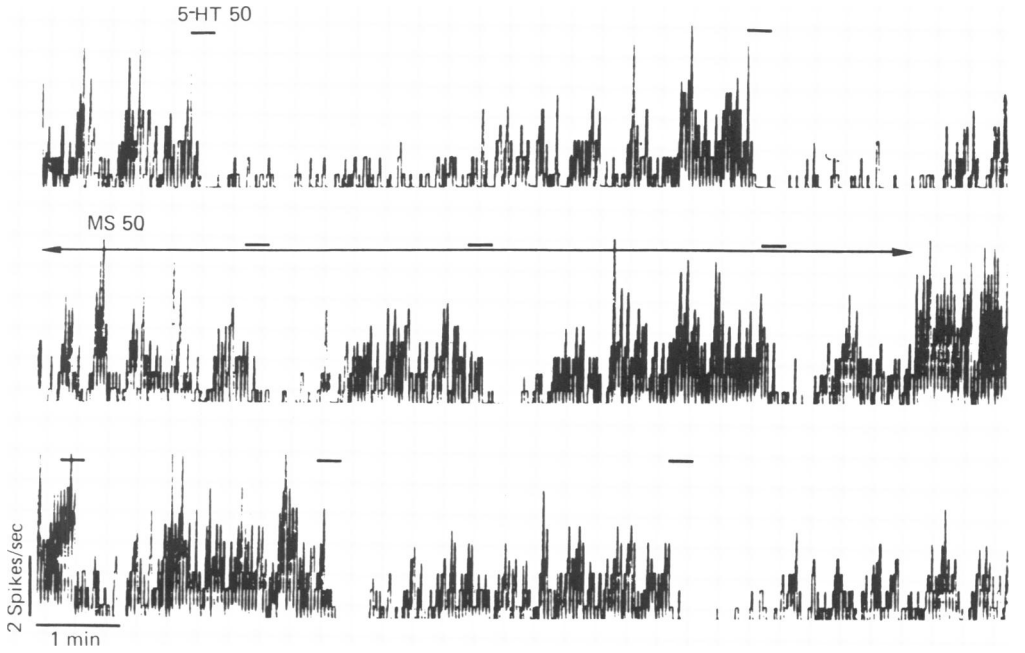
towards spontaneous locus coeruleus activity (Text-fig. 6) which prohibited the use of large ejection currents. Also, it is possible that dorsal raphe action is exerted at least partly on dendrites which are remote from the pipette. For that reason the effects of methysergide towards the inhibitory action of dorsal raphe stimula-



Text-fig. 6. A partial antagonistic action of methysergide (MS) applied ionophoretically on induced suppression of spontaneous locus coeruleus activity. The six records (*A–F*) are continuous. Dorsal raphe stimulation is applied at regular intervals for the duration indicated by the bar underneath each record. MS current (25 nA) was turned on before *B* and turned off after *D*. Note the rather high frequency but irregular firing of the neurone. MS in this case did not appreciably affect spontaneous cellular activity.

tion on locus coeruleus excitatory responses to leg pinch were not tested systematically.

When applied ionophoretically, serotonin exerted a long lasting suppression of spontaneous activity of ten locus coeruleus neurones (Text-fig. 7) tested. This suppression was reversibly antagonized by concurrent ionophoretic administration of methysergide (six of ten cells tested, Text-fig. 7).



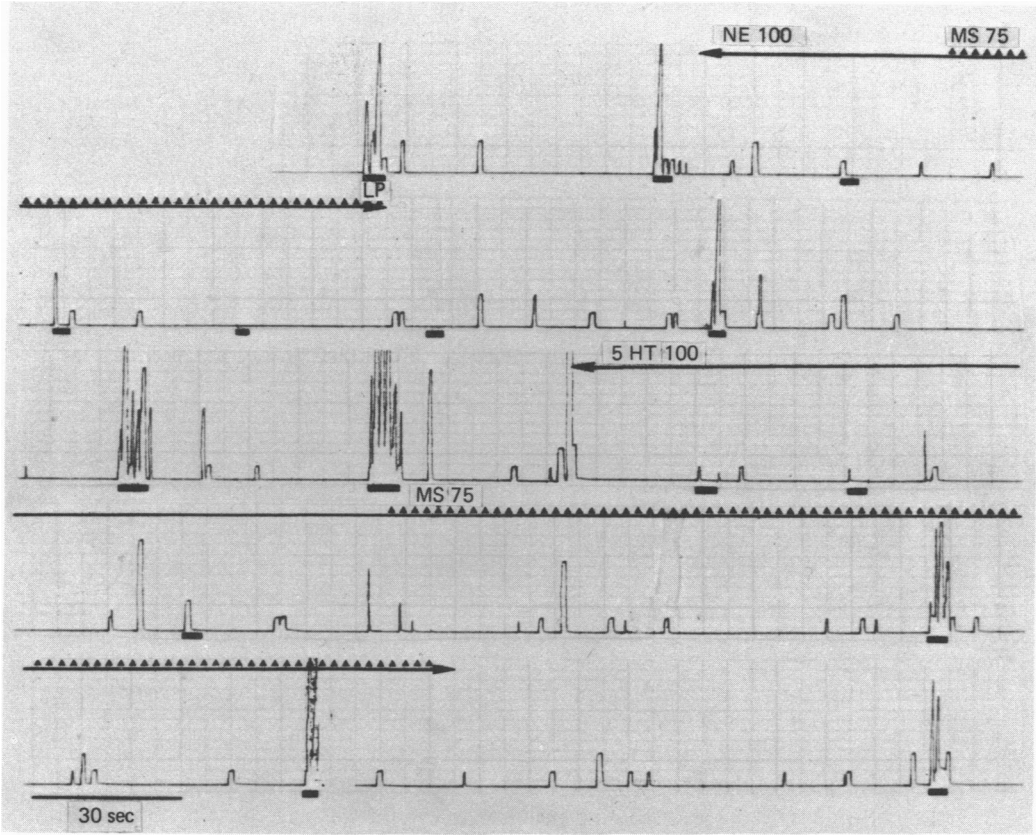
Text-fig. 7. The effects of serotonin (5-HT) applied ionophoretically (50 nA) on spontaneous activity of a locus coeruleus neurone. 5-HT induced a long lasting inhibition of locus coeruleus activity but that effect was greatly reduced during and a few minutes after the concurrent ionophoresis of methysergide (MS, 50 nA).

During the application of serotonin, there was a marked reduction in the magnitude of the cellular responses to the leg pinch in six cells tested (Text-fig. 8). A similar reduction in magnitude of excitatory responses was seen in these same cells during the ionophoresis of norepinephrine. This reduction in responsiveness is expected in view of the recent description by Aghajanian, Cedarbaum & Wang (1977) of a powerful norepinephrine mediated collateral inhibition of locus coeruleus neurones. The serotonin-mediated inhibition could be dissociated from that produced by norepinephrine; only the former was reversible by methysergide (four of six cells tested) whereas norepinephrine-mediated inhibition was resistant to the blocking action of methysergide in all six cells tested.

To further test the hypothesis that dorsal raphe stimulation is mediated by serotonin, rats (n = 5) were treated with PCPA to block the synthesis of serotonin (Koe & Weissman, 1966). In five of eight locus coeruleus cells in these rats, tested 3–5 days after the injection of PCPA, dorsal raphe stimulation was no longer effective in reducing locus coeruleus excitatory responses to the noxious stimulus (Text-fig 9).

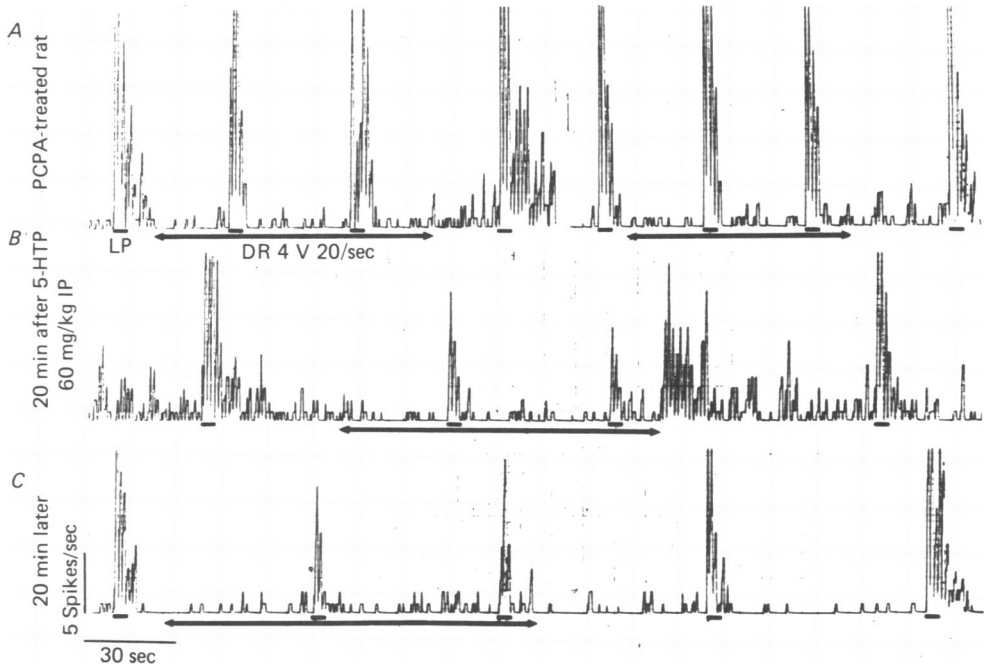
Furthermore an injection of 5-HTP, which temporarily restores serotonin synthesis in the brain, restored the efficacy of dorsal raphe stimulation in inhibiting both spontaneous and noxious-stimulus-evoked locus coeruleus activity (Text-fig. 9). It should be added that PCPA treatment did not alter the efficacy of morphine in reducing the excitatory responses of locus coeruleus cells to leg pinch.

An injection of the neurotoxin, 5,7-DHT is reported to cause destruction of cell bodies and terminals containing serotonin (Baumgarten, Björklund, Lachenmayer & Nobin, 1973). Four rats were injected into the dorsal raphe with 5,7-DHT and tested

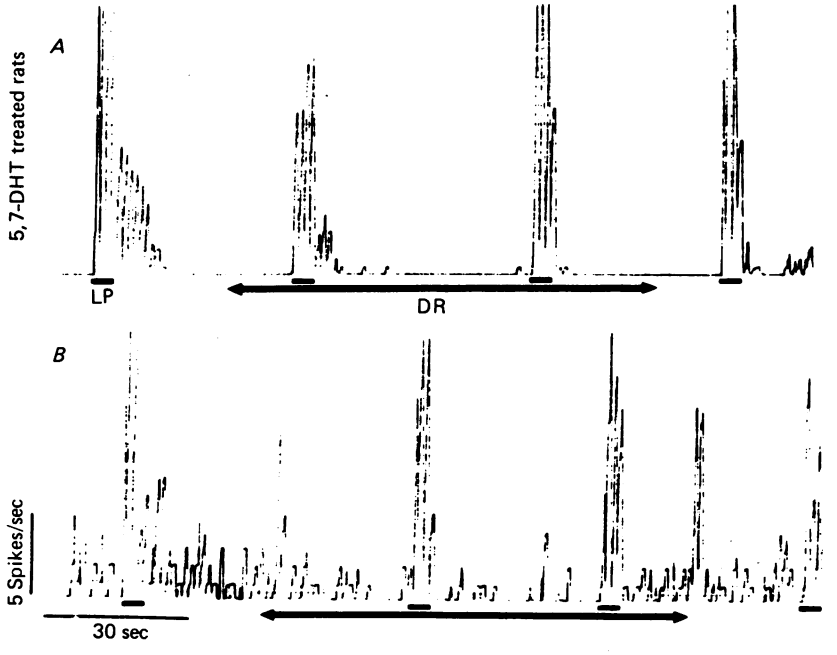


Text-fig. 8. Blockade of excitatory locus coeruleus cellular responses to a leg pinch (LP) during the ionophoresis of norepinephrine (NE) or serotonin (5-HT). Concurrent application of methysergide (MS) does not overcome the effects of norepinephrine but does reduce the effects of 5-HT.

3–6 days later. All eight locus coeruleus cells studied in these rats reacted with a typical excitatory response to a leg-pinch. As seen after PCPA pretreatment, morphine was still capable of blocking this excitatory response (three cells tested) and naloxone reversed morphine effects. In none of the four rats tested did dorsal raphe stimulation affect in any way the excitatory locus coeruleus responses to the noxious stimulus. An occasional effect of dorsal raphe stimulation towards spontaneous locus coeruleus activity could still be seen (Text-fig 10).



Text-fig. 9. The effects of blockade of serotonin synthesis with PCPA on the efficacy of the dorsal raphe in suppression of locus coeruleus excitatory responses to leg pinch. The responses of such a cell to leg pinch in PCPA-treated rats was not affected by the pharmacological treatment yet the efficacy of dorsal raphe stimulation was blocked by the drug treatment. However, upon injection of the serotonin precursor 5-HTP, dorsal raphe stimulation regained its efficacy.



Text-fig. 10. Lack of effects of the dorsal raphe towards locus coeruleus excitatory responses in rats injected with 5,7-DHT, which destroys serotonin terminals. Results from two rats (*A* and *B*) are presented.

DISCUSSION

The results of the present study confirm earlier observations that locus coeruleus neurones react to noxious stimuli and that these responses are morphine sensitive. An inhibitory pathway between the dorsal raphe nucleus and the locus coeruleus is described and it is suggested that the pathway uses serotonin as a neurotransmitter. Furthermore, it is proposed that the behavioural antinociceptive action of dorsal raphe stimulation is exerted, among other possible sites, in the nucleus locus coeruleus.

Although the complete afferent connexions of the locus coeruleus are as yet undisclosed, the present results corroborate earlier suggestions that such cells may receive an input from nociceptive fibres, as shown by their responses to noxious stimuli. Correlated with this effect is the presence of high concentrations of morphine receptors as well as enkephalins in the locus coeruleus (Pert *et al.* 1975), and, as shown in the present investigation, the high sensitivity of locus coeruleus cells to both parenteral and ionophoretic application of morphine and the antagonistic action of naloxone whether applied parenterally or by ionophoresis. The coherence between effects of the two modes of morphine administration indicated that systemic morphine may indeed act directly on locus coeruleus cells. It is not part of the 'traditional' pain system in the brain, and therefore, the functional significance of such nocioceptive responses is, presently, only subject to speculation.

Several lines of evidence suggest the existence of a serotonin-mediated connexion between the dorsal raphe and the locus coeruleus. (1) There is an anatomical connexion between them, described with both anterograde (Taber-Pierce *et al.* 1976) and retrograde (Sakai *et al.* 1977) tracing methods. (2) Stimulation of the dorsal raphe with relatively low currents produces inhibition of locus coeruleus spontaneous and pain-evoked activity. (3) A similar inhibition is exerted as a response to ionophoretic application of 5-HT. (4) The effects of drugs which blocks synthesis (PCPA) or destroy terminals (5,7-DHT) are coherent with the serotonergic nature of the connexion between the dorsal raphe and the locus coeruleus. Admittedly, the relatively weak effects of the serotonin antagonist methysergide towards both dorsal raphe and 5-HT induced locus coeruleus inhibition is bothersome, yet serotonin antagonists including methysergide exert only partial (Segal, 1976*b*) or even minor (Haigler & Aghajanian, 1974) effects elsewhere. Taken together, the present results do suggest the existence of an inhibitory serotonergic synapse of raphe origin in the locus coeruleus.

The dorsal raphe exerts a potent antagonistic action towards locus coeruleus excitatory responses to noxious stimuli. This effect is much stronger than the effects of dorsal raphe stimulation on spontaneous locus coeruleus activity. This difference between dorsal raphe effects on spontaneous and evoked locus coeruleus activity may indicate at least a partial presynaptic action of serotonin on incoming pain carrying fibres. A presynaptic action on nociceptor fibres in the spinal cord has been attributed to enkephalin-containing fibres (Lamotte, Pert & Snyder, 1976). It was therefore imperative to test the possibility that morphine and dorsal raphe stimulation might share a final common path. Although both block locus coeruleus responses to noxious stimuli, it is unlikely that dorsal raphe stimulation and morphine have an identical site of action towards locus coeruleus neurones; naloxone did not affect the in-

hibitory action of dorsal raphe stimulation towards evoked locus coeruleus activity and vice versa (PCPA and 5,7-DHT did not cause significant changes in the efficacy of morphine). Indirect evidence supports this contention; morphine does not affect the activity of dorsal raphe neurones (Haigler, 1976). If indeed morphine would act presynaptically on serotonin synapses, one would expect to detect changes in dorsal raphe cellular activity after parenteral morphine administration due to a probable feed-back loop (Aghajanian, 1972). The independence of dorsal raphe stimulation effects towards locus coeruleus activity from the effects of morphine along with the lack of effects of morphine on dorsal raphe activity renders it difficult to explain the analgesic properties of the micro-injection of morphine in the periaquaductal area in terms of an action on dorsal raphe neurones. Although these effects are by no means restricted to the dorsal raphe area but concentrated in more lateral aspects of the periaquaductal grey, they do suggest a local effect of morphine, which is different from the present suggestion of a remote site of action of dorsal raphe stimulation (e.g. in the locus coeruleus and elsewhere). The recent proposal that morphine acts via a descending medullary raphe-spinal pathway represents a different view of pain control. However, the possible contribution of a direct locus coeruleus-spinal projection (Nygren & Olson, 1977) in the control of noxious input remains to be considered in conjunction with the influence of the dorsal raphe on the locus coeruleus.

The possible reciprocal connexion between the serotonergic and noradrenergic system in the brain, have been the subject of numerous theories and experiments (Kostowski, 1975). It has long been suggested, based largely on indirect data, that norepinephrine and serotonin exert opposite actions towards several brain functions. Indeed, fibres of locus coeruleus and raphe origin seem to share many terminal areas in the brain. The present results suggest that these opposing actions are not necessarily exerted at the common terminal areas; activation of the serotonergic system may directly inhibit noradrenergic cellular activity. It is not clear as yet if the reverse is true also. Nevertheless, the monosynaptic connexions between the locus coeruleus and the raphe nuclei, between the dorsal and median raphe (Mosko, Haubrich & Jacobs, 1977) and between the raphe nuclei and the dopaminergic substantia nigra (Dray, Gonye, Oakley & Tanner, 1976) indicate that the monoamines in the brain are functionally interdependent. Further studies are expected to untangle these complex functional connexions of the monoamines.

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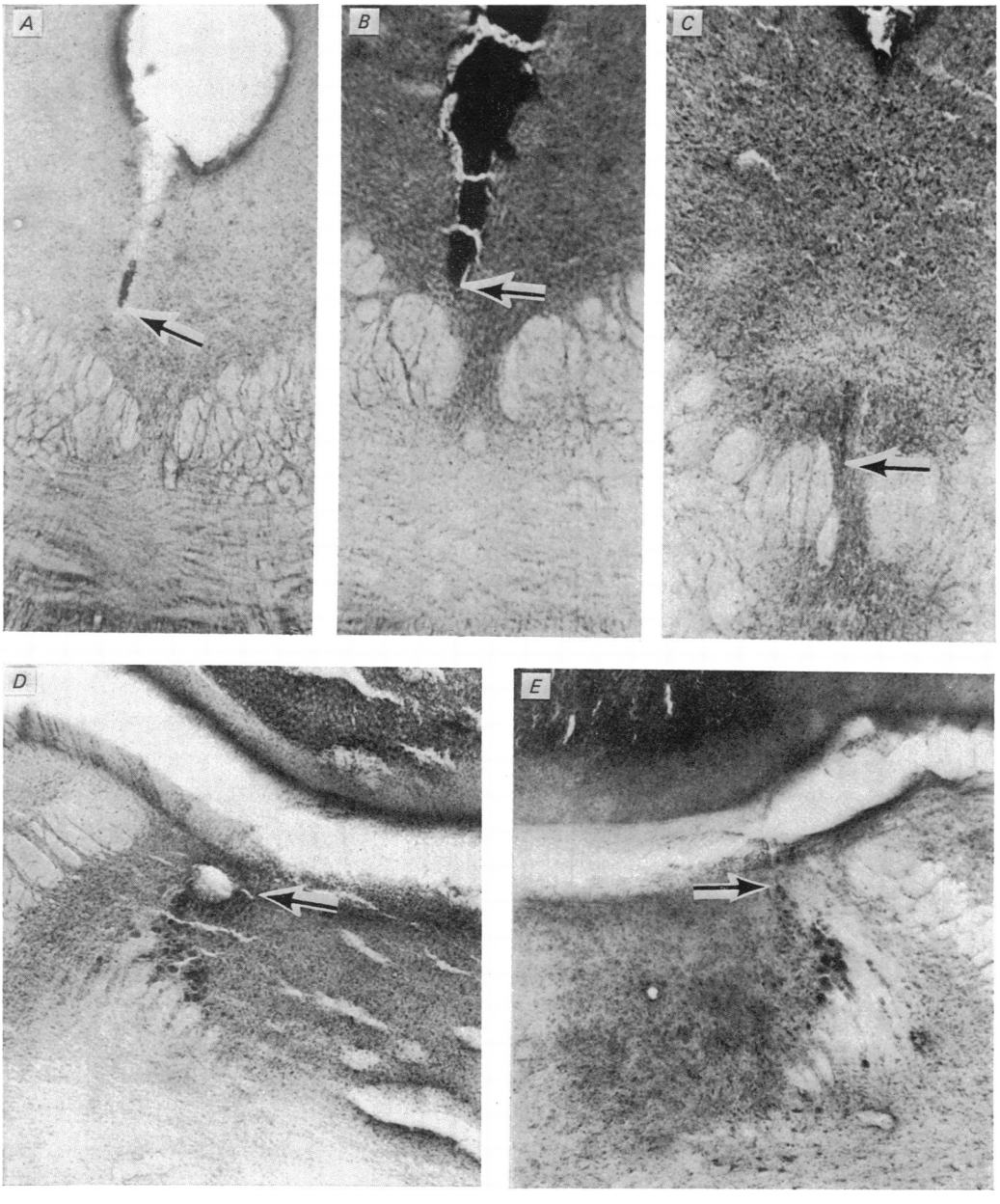
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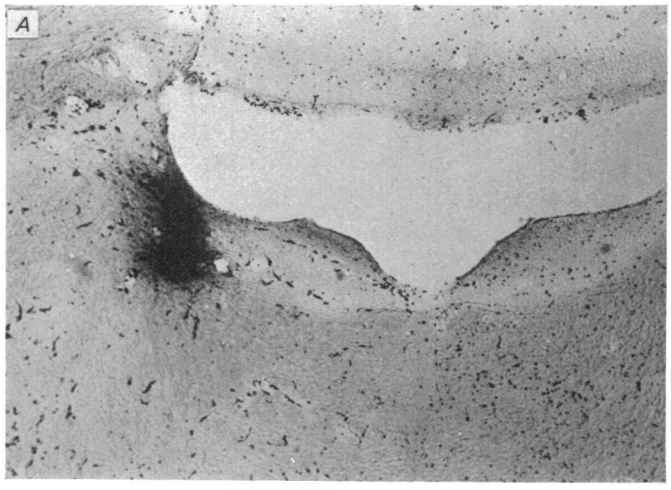
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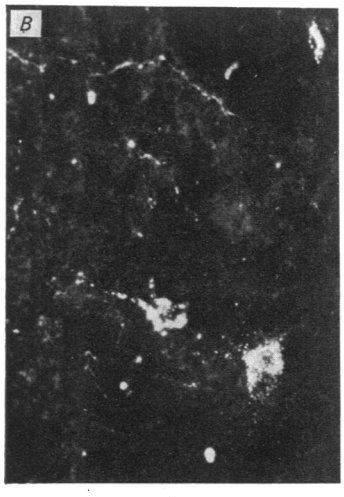
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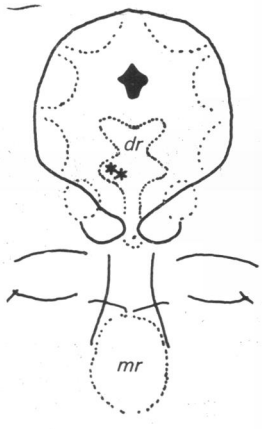
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EXPLANATION OF PLATES

PLATE 1

Localization of stimulating (*A*, *B*, *C*) and recording (*D*, *E*) electrodes in the dorsal raphe and the locus coeruleus, respectively. The arrows indicate the electrode tips in all examples. The sections were stained with cresyl violet and electrode penetrations were traced on serial sections. Magnification 25 ×.

PLATE 2

Labelling of dorsal raphe neurones by horseradish peroxidase after an ionophoretic injection of the enzyme into the locus coeruleus. *A*, injection site, unstained section 25 ×. *B*, examples of two labelled neurones 200 ×. *C*, schematic diagram of the region where the labelled cells were found. Asterisks indicate labelled neurones.